

IN THE CLAIMS

Please amend the claims as follows:

Claim 1 (Withdrawn): A method of producing a biosensor protein capable of regulating a fluorescence property of green fluorescent protein or its derivative by modifying the structure of green fluorescent protein or its derivative, comprising the steps of:

(A) predicting a hotspot amino acid residue affecting a fluorescence property of green fluorescent protein or its derivative;

(B) producing various fusion proteins which have the structure linked with a modified fluorescent protein and one or more functional molecules,

the modified fluorescent protein being the protein obtained by cleaving amino acid sequence of green fluorescent protein or its derivative in the vicinity of the hotspot amino acid residue and modifying the structure of green fluorescent protein or its derivative, and

the functional molecules each being the molecules capable of transmitting their conformational changes to the modified fluorescent protein to cause a conformational change of the modified fluorescent protein, thereby altering the fluorescence property of the modified fluorescent protein;

(C) reacting the resultant various fusion proteins with a factor affecting the conformation of any of the functional molecules; and

(D) screening a fusion protein exhibiting a change in the fluorescence property by the reaction of the step (C), as a biosensor protein, from the various fusion proteins.

Claim 2 (Withdrawn): The method according to claim 1, wherein the fluorescence property is fluorescence intensity.

Claim 3 (Withdrawn): A biosensor protein comprising (1) and (2) below:

(1) a modified fluorescent protein which is obtained by cleaving amino acid sequence of green fluorescent protein or its derivative in the vicinity of a hotspot amino acid residue which affects the fluorescence property, and modifying the structure of the green fluorescent protein or its derivative; and

(2) one or more functional molecules which are capable of transmitting their conformational changes to the modified fluorescent protein to cause a conformational change of the modified fluorescent protein, thereby altering the fluorescence property of the modified fluorescent protein.

Claim 4 (Withdrawn): The biosensor protein according to claim 3, wherein the fluorescence property is fluorescence intensity.

Claim 5 (Withdrawn): The biosensor protein according to claim 3, wherein the hotspot amino acid residue corresponds to the 148th amino acid of amino acid sequence of green fluorescent protein.

Claim 6 (Withdrawn): The biosensor protein according to claim 4, wherein the hotspot amino acid residue corresponds to the 148th amino acid of amino acid sequence of green fluorescent protein.

Claim 7 (Withdrawn): The biosensor protein according to claim 3, wherein the hotspot amino acid residue corresponds to the 94th amino acid of amino acid sequence of green fluorescent protein.

Claim 8 (Withdrawn): The biosensor protein according to claim 4, wherein the hotspot amino acid residue corresponds to the 94th amino acid of amino acid sequence of green fluorescent protein.

Claim 9 (Withdrawn): The biosensor protein according to claim 3, wherein the hotspot amino acid residue corresponds to the 96th amino acid of amino acid sequence of green fluorescent protein.

Claim 10 (Withdrawn): The biosensor protein according to claim 4, wherein the hotspot amino acid residue corresponds to the 96th amino acid of amino acid sequence of green fluorescent protein.

Claim 11 (Withdrawn): The biosensor protein according to claim 3, wherein the hotspot amino acid residue corresponds to the 222nd amino acid of amino acid sequence of green fluorescent protein.

Claim 12 (Withdrawn): The biosensor protein according to claim 4, wherein the hotspot amino acid residue corresponds to the 222nd amino acid of amino acid sequence of green fluorescent protein.

Claim 13 (Withdrawn): A biosensor protein comprising (1) and (2) below:

(1) a modified green fluorescent protein having the following amino acid sequences (a) and (b) in this order from the N terminus:

- (a) an amino acid sequence of X-th to 238th position of green fluorescent protein; and
- (b) an amino acid sequence of 1st to Y-th position of green fluorescent protein, (where X is an arbitrary number from 148 to 150, and Y is an arbitrary number from 140 to 147); and
- (2) one or more functional molecules which are capable of transmitting their conformational changes to the modified green fluorescent protein to cause a conformational change of the modified green fluorescent protein, thereby altering the fluorescence property of the modified green fluorescent protein.

Claim 14 (Withdrawn): A biosensor protein comprising the following sequences (a) to (h) sequentially from the N terminus:

- (a) an amino acid sequence containing methionine (linker X);
- (b) myosin light chain kinase protein or a partial amino acid sequence thereof;
- (c) an amino acid sequence (linker Y) for linking the above sequence (b) and the following sequence (d);
- (d) an amino acid sequence from X-th to 238<sup>th</sup> position of green fluorescent protein (where X is an arbitrary number from 148 to 150);
- (e) an amino acid sequence for linking the above sequence (d) and the following sequence (f);
- (f) an amino acid sequence from 1st to Y-th position of green fluorescent protein (where Y is an arbitrary number from 140 to 147);
- (g) an amino acid sequence (linker Z) for linking the above sequence (f) and the following sequence (h); and
- (h) calmodulin protein or a partial amino acid sequence thereof.

Claim 15 (Withdrawn): A biosensor protein comprising (1) and (2) below:

(1) a modified green fluorescent protein having the following amino acid sequences (a) and (b) in this order from the N terminus:

(a) the amino acid sequence from the 149th to 238th position of green fluorescent protein, and

(b) the amino acid sequence from the 1st to 144th position of green fluorescent protein; and

(2) one or more functional molecules which are capable of transmitting their conformational changes to the modified green fluorescent protein to cause a conformational change of the modified green fluorescent protein, thereby altering the fluorescence property of the modified green fluorescent protein.

Claim 16 (Withdrawn): A biosensor protein comprising the following sequences (a) to (h) sequentially from the N terminus:

(a) an amino acid sequence containing methionine (linker X);

(b) myosin light chain kinase protein or a partial amino acid sequence thereof;

(c) an amino acid sequence (linker Y) for linking the above sequence (b) and the following sequence (d);

(d) the amino acid sequence from the 149th to 238th position of green fluorescent protein;

(e) an amino acid sequence for linking the above sequence (d) and the following sequence (f);

(f) the amino acid sequence from the 1st to 144th position of green fluorescent protein;

(g) an amino acid sequence (linker Z) for linking the above sequence (f) and the following sequence (h); and

(h) calmodulin protein or a partial amino acid sequence thereof.

Claim 17 (Withdrawn): The biosensor protein according to claim 3, wherein the functional molecules are calmodulin protein or a partial amino acid sequence thereof, and myosin light chain kinase protein or a partial amino acid sequence thereof.

Claim 18 (Withdrawn): The biosensor protein according to claim 4, wherein the functional molecules are calmodulin protein or a partial amino acid sequence thereof, and myosin light chain kinase protein or a partial amino acid sequence thereof.

Claim 19 (Withdrawn): The biosensor protein according to claim 5, wherein the functional molecules are calmodulin protein or a partial amino acid sequence thereof, and myosin light chain kinase protein or a partial amino acid sequence thereof.

Claim 20 (Withdrawn): The biosensor protein according to claim 6, wherein the functional molecules are calmodulin protein or a partial amino acid sequence thereof, and myosin light chain kinase protein or a partial amino acid sequence thereof.

Claim 21 (Withdrawn): The biosensor protein according to claim 7, wherein the functional molecules are calmodulin protein or a partial amino acid sequence thereof, and myosin light chain kinase protein or a partial amino acid sequence thereof.

Claim 22 (Withdrawn): The biosensor protein according to claim 8, wherein the functional molecules are calmodulin protein or a partial amino acid sequence thereof, and myosin light chain kinase protein or a partial amino acid sequence thereof.

Claim 23 (Withdrawn): The biosensor protein according to claim 9, wherein the functional molecules are calmodulin protein or a partial amino acid sequence thereof, and myosin light chain kinase protein or a partial amino acid sequence thereof.

Claim 24 (Withdrawn): The biosensor protein according to claim 10, wherein the functional molecules are calmodulin protein or a partial amino acid sequence thereof, and myosin light chain kinase protein or a partial amino acid sequence thereof.

Claim 25 (Withdrawn): The biosensor protein according to claim 11, wherein the functional molecules are calmodulin protein or a partial amino acid sequence thereof, and myosin light chain kinase protein or a partial amino acid sequence thereof.

Claim 26 (Withdrawn): The biosensor protein according to claim 12, wherein the functional molecules are calmodulin protein or a partial amino acid sequence thereof, and myosin light chain kinase protein or a partial amino acid sequence thereof.

Claim 27 (Withdrawn): The biosensor protein according to claim 13, wherein the functional molecules are calmodulin protein or a partial amino acid sequence thereof, and myosin light chain kinase protein or a partial amino acid sequence thereof.

Claim 28 (Withdrawn): The biosensor protein according to claim 15, wherein the functional molecules are calmodulin protein or a partial amino acid sequence thereof, and myosin light chain kinase protein or a partial amino acid sequence thereof.

Claim 29 (Withdrawn): A biosensor protein comprising the following sequences (a) to (h) sequentially from the N terminus:

- (a) Met-Gly-Thr or Met-Val-Asp (linker X);
- (b) a partial amino acid sequence of myosin light chain kinase protein ISer-Ser-Arg-Arg-Lys-Trp-Asn-Lys-Thr-Gly-His-Ala-Val-Arg-Ala-Ile-Gly-Arg-Leu-Ser-Ser); [SEQ ID NO:6]
- (c) Leu-Glu (linker Y);
- (d) the amino acid sequence from the 149th to 238th position of green fluorescent protein;
- (e) Gly-Gly-Thr-Gly-Gly-Ser (linker amino acid sequence; amino acids 117 to 122 of SEQ ID NO:8);
- (f) the amino acid sequence from the 1st to 144th position of green fluorescent protein;
- (g) Gly-Thr-Arg or Thr-Arg (linker Z); and
- (h) the amino acid sequence from the 2nd to 148th position of rat calmodulin protein.

Claim 30 (Withdrawn): A biosensor protein comprising the following sequences (a) to (c) in this order from the N terminus:

- (a) the amino acid sequence from the 1st to 144th position of green fluorescent protein;



(b) one or more functional molecules which are capable of transmitting their conformational changes to the modified green fluorescent protein to cause a conformational change of the modified green fluorescent protein, thereby altering the fluorescence property of the modified green fluorescent protein; and

(c) the amino acid sequence from the 149th to 238th position of green fluorescent protein.

Claim 31 (Withdrawn): The biosensor protein according to claim 30, wherein the functional molecules are calmodulin protein or a partial amino acid sequence thereof, and myosin light chain kinase protein or a partial amino acid sequence thereof.

Claim 32 (Withdrawn): A biosensor protein comprising the following sequences (a) to (g) sequentially from the N terminus:

(a) the amino acid sequence from the 1st to 144th position of green fluorescent protein;

(b) an amino acid sequence (linker A) for linking the above sequence (a) and the following sequence (c);

(c) calmodulin protein or a partial amino acid sequence thereof;

(d) an amino acid sequence (linker B) for linking the above sequence (c) and the following sequence (e);

(e) myosin light chain kinase protein or a partial amino acid sequence thereof;

(f) an amino acid sequence (linker C) for linking the above sequence (e) and the following sequence (g); and

(g) the amino acid sequence from the 149th to 238th position of green fluorescent protein.

Claim 33 (Withdrawn): A biosensor protein comprising the following sequences (a) to (g) sequentially from the N terminus: (a) the amino acid sequence from the 1st to 144th position of green fluorescent protein;

(b) Gly-Thr-Arg (linker A);

(c) the amino acid sequence from the 2nd to 148th position of rat calmodulin protein;

(d) Gly-Thr or [Gly-Thr-Gly-Ser-Gly-Gly-Gly-Sere (linker B; SEQ ID NO:17);

(e) a partial amino acid sequence of myosin light chain kinase protein (Ser-Ser-Arg-Arg-Lys-Trp-Asn-Lys-Thr-Gly-His-Ala-Val-Arg-Ala-Ile-Gly-Arg-Leu-Ser-Ser); [SEQ ID NO:6];

(f) Thr-Ser (linker C);

(g) the amino acid sequence from the 149th to 238th position of green fluorescent protein.

Claims 34-59 (Canceled).

Claim 60 (Currently Amended): A nucleic acid molecule encoding an artificial biosensor protein ~~A biosensor gene encoding the biosensor protein according to claim 29~~

comprising the following sequences (a) to (h) sequentially from the N terminus:

(a) Met-Gly-Thr or Met-Val-Asp (linker X);

(b) a partial amino acid sequence of myosin light chain kinase protein (Ser-Ser-Arg-Arg-Lys-Trp-Asn-Lys-Thr-Gly-His-Ala-Val-Arg-Ala-Ile-Gly-Arg-Leu-Ser-Ser)(SEQ ID NO: 6);

(c) Leu-Glu (linker Y);

(d) the amino acid sequence from the 149th to 238th position of green fluorescent protein of SEQ ID NO: 2;

(e) Gly-Gly-Thr-Gly-Gly-Ser (linker amino acid sequence (amino acids 117 to 122 of SEQ ID NO: 8);

(f) the amino acid sequence from the 1st to 144th position of green fluorescent protein of SEQ ID NO: 2;

(g) Gly-Thr-Arg or Thr-Arg (linker Z); and

(h) the amino acid sequence from the 2nd to 148th position of rat calmodulin protein of SEQ ID NO: 4 or the amino acid sequence from the 2<sup>nd</sup> to the 148<sup>th</sup> position of calmodulin protein mutant CaMCN.

Claims 61-63 (Canceled).

Claim 64 (Currently Amended): A nucleic acid molecule encoding an artificial biosensor protein ~~A biosensor gene encoding the biosensor protein according to claim 33~~

comprising the following sequences (a) to (g) sequentially from the N terminus:

(a) the amino acid sequence from the 1st to 144th position of green fluorescent protein or SEQ ID NO: 2;

(b) Gly-Thr-Arg (linker A);

(c) the amino acid sequence from the 2nd to 148th position of rat calmodulin protein of SEQ ID NO: 2;

(d) Gly-Thr or Gly-Thr-Gly-Ser-Gly-Gly-Gly-Sere (linker B; SEQ ID NO: 17);

(e) a partial amino acid sequence of myosin light chain kinase protein (Ser-Ser-Arg-Arg-Lys-Trp-Asn-Lys-Thr-Gly-His-Ala-Val-Arg-Ala-Ile-Gly-Arg-Leu-Ser-Ser)(SEQ ID NO: 6);

(f) Thr-Ser (linker C);

(g) the amino acid sequence from the 149th to 238th position of green fluorescent protein of SEQ ID NO: 2.

Claim 65 (New): A nucleic acid molecule encoding an artificial biosensor protein comprising the following sequences (a) to (h) sequentially from the N terminus:

(a) Met-Gly-Thr or Met-Val-Asp (linker X);

(b) a partial amino acid sequence of myosin light chain kinase protein (Ser-Ser-Arg-Arg-Lys-Trp-Asn-Lys-Thr-Gly-His-Ala-Val-Arg-Ala-Ile-Gly-Arg-Leu-Ser-Ser) (SEQ ID NO: 6);

(c) an amino acid sequence that is selected from the group consisting of Thr-Ser, Gly-Ser, Leu-Glu, Thr-Tyr, Thr-Asp, Thr-Cys, Thr-Phe, Thr-Met, Thr-Thr, Thr-Glu, Thr-His and Thr-Leu (linker Y);

(d) the amino acid sequence from the 149th to 238th position of green fluorescent protein of SEQ ID NO: 2;

(e) Gly-Gly-Thr-Gly-Gly-Ser (linker amino acid sequence; amino acids 117 to 122 of SEQ ID NO: 8);

(f) the amino acid sequence from the 1st to 144th position of green fluorescent protein of SEQ ID NO: 2;

(g) Gly-Thr-Arg or Thr-Arg (linker Z); and

(h) the amino acid sequence from the 2nd to 148th position of rat calmodulin protein of SEQ ID NO: 4 or the amino acid sequence from the 2nd to 148th position of calmodulin protein mutant CaMCN.

Claim 66 (New): A nucleic acid molecule encoding an artificial biosensor protein comprising the following sequences (a) to (h) sequentially from the N terminus:

(a) Met-Gly-Thr or Met-Val-Asp (linker X);

(b) a partial amino acid sequence of myosin light chain kinase protein (Ser-Ser-Arg-Arg-Lys-Trp-Asn-Lys-Thr-Gly-His-Ala-Val-Arg-Ala-Ile-Gly-Arg-Leu-Ser-Ser) [SEQ ID NO: 6];

(c) an amino acid sequence that is selected from the group consisting of Thr-Ser, Gly-Ser, Leu-Glu, Thr-Tyr, Thr-Asp, Thr-Cys, Thr-Phe, Thr-Met, Thr-Thr, Thr-Glu, Thr-His and Thr-Leu (linker Y);

(d) an amino acid sequence from X-th to 238th position of green fluorescent protein of SEQ ID NO: 2 (where X is an arbitrary number from 148 to 150);

(e) Gly-Gly-Thr-Gly-Gly-Ser (linker amino acid sequence; amino acids 117 to 122 of SEQ ID NO: 8);

(f) an amino acid sequence from 1st to Y-th position of green fluorescent protein of SEQ ID NO: 2 (where Y is 140 when X is 148, Y is 144 or 147 when X is 149, or Y is 144 or 147 when X is 150);

(g) Gly-Thr-Arg or Thr-Arg (linker Z); and

(h) the amino acid sequence from the 2<sup>nd</sup> to 148<sup>th</sup> position of rat calmodulin protein of SEQ ID NO: 4 or the amino acid sequence from the 2<sup>nd</sup> to 148<sup>th</sup> position of calmodulin protein mutant CaMCN.